

Microwave-Assisted Stabilisation and Storage Stability Study of Rice Bran Oil from Different Varieties

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Rice bran is a by-product from milling process of paddy rice to produce refined rice, however until today, there was limited utilisation of rice bran even though rice bran has the ability and potential to become of the major supplementary sources that function as food agent substance. This is due to the instability of rice bran as it will become unstable due to the hydrolysis and oxidation process. The oxidation process, mainly due to the oil presence in the rice bran, releases substances that is unsuitable for consumption. Immediate extraction of the oil from the rice bran is needed as it will be hydrolysed into free fatty acids (FFA) and glycerol by the activity of lipase enzymes. The stabilisation of rice bran can be done by controlling the lipase activity. This process could indirectly prevent the rice bran from being oxidised. Studies have shown that the lipase enzymes in the rice bran can be inactivated depends on the temperature and duration of heat exposure (stabilisation) as well as the moisture content. In this study, the effect of microwave-assisted stabilisation of rice bran was investigated on the quality of rice bran oil (RBO) extracted. Different varieties of rice bran were used in this study which was from lowland, upland and Bario. Extraction of the rice bran was performed using Soxhlet extractor and quality of RBO was determined by analysing its properties in terms of crude RBO yield, moisture content and free fatty acid content. Storage stability of the rice bran was evaluated until 40 d. The results demonstrated that the Bario rice bran showed decrease of oil yield from 0 - 40 d with a difference of 48.9 %, followed by the lowland rice bran by 48.7 %. The upland rice bran oil gives the higher different percentage of 64.2 % during the storage. An increase of moisture content was observed during the storage for all stabilised and unstabilised rice brans. Free fatty acid contents show increase amount in almost all types of rice bran range from 2.5 to 11.9 %.

1. Introduction

Microwave stabilisation occurs when alternating current is passed through a food sample, and heat is generated by virtue of the sample's electrical resistance. A large number of potential future applications exist for microwave technique, including its use in evaporation, dehydration, fermentation, and extraction. Previous studies have shown the importance of microwave heating in conjunction with extraction processes. The extractions using microwave heating have used minimal heat to moderate amounts of lipids and high moisture contents. No recognised studies have dealt with microwave heating and subsequent extraction of low moisture.

Rice bran is a component of raw rice that is obtained when it is removed from the starchy endosperm in the rice milling process. It has high oil content (15 - 25 %), low moisture content (6 - 7 %) and possesses a powdery consistency (Saunders, 1990). Rice bran is a waste product in the milling process; it has been used as a feedstock and has the potential to be used as a food ingredient and oil source (McCaskill and Zhang, 1999). However, it must be stabilised immediately upon production due primarily to the presence of lipase, an

enzyme that rapidly hydrolyses oil to free fatty acids (FFA) and glycerol, and results in a drastic quality reduction of the rice bran (Malekian et al., 2000).

The primary means for rice bran stabilisation include deactivating the enzyme through heat treatment such as extrusion or microwave heating (Zigoneanu et al., 2008). Up till now, there is no known literature involving the use of ohmic heating as a potential method for rice bran stabilisation. Another promising use of rice bran involves rice bran oil that can be extracted from rice bran. Several studies have characterized rice bran oil's superior cooking, nutritional, and sensory properties and have also explained the lack of widespread commercial use of rice bran oil due to economic factors (McCaskill and Zhang, 1999). Developing economic methods to stabilise rice bran and extract rice bran oil are important. The objective of this study was to investigate the effect of microwave-assisted stabilisation of rice bran on the quality of rice bran oil (RBO) extracted.

2. Material and methods

Three types of rice bran (Lowland, Upland, Bario) were used for the extraction of rice bran oil. Lowland rice bran was purchased from Faiza Sdn. Bhd. Upland rice was collected from farmers at Kampung Charok Tok Pong, Baling, Kedah named as Sukhai Hitam, while Bario rice was collected from farmers in Bario, Sarawak. Methanol, N-Hexane and ethanol solvent were supplied by Relab Scientific Sdn. Bhd. The phenolphthalein reagent was purchased from Riedel De-Haen, Sigma-Aldrich and sodium hydroxide (NaOH) and potassium hydroxide (KOH) were supplied by E. Merck. This section represented the steps in obtaining crude oil yield from rice bran, including sample preparation, sample stabilisation, sample analysis and sample extraction. Weigh of loss by heating method is used to determine the amount of crude oil extracted from rice bran. The extraction and determination of volatile fatty acid is carried out by Soxtherm extractor.

2.1 Preparation of rice bran

The fresh rice was milled using a milling machine; brand Satake at Faculty of Engineering, Universiti Putra Malaysia. The milled rice bran was then screened to pass through a 710 μm aperture sieve to remove the broken grains, hull fragments, paddy kernels and foreign materials. The rice bran had been stored in sealed polyethylene bags and kept in refrigerator at 0 °C in order to control the growth of free fatty acid (FFA) in the rice bran (Mohd Daud et al., 2016).

2.2 Microwave stabilisation of rice bran

The stabilisation procedure was carried out for all samples, which included the lowland, upland and bario rice bran according to the method of Malekian et al. (2000) but with some modifications. The stabilisation of rice bran was performed by using the microwave oven with 1,000 W output power. Hundred grams of each sample was heated in a pre-heated microwave oven for 3 min at 40 % power. The moisture content of raw rice bran was adjusted from an original value into a 21 % through the addition of water (Malekian et al., 2000). The sample was mixed thoroughly to ensure the water was evenly distributed before undergoing the heating procedure.

The rice brans were cooled down at room temperature overnight. This procedure was repeated three times for all samples to make sure the stabilisation of rice bran. All samples were placed in a chiller at 4 °C until analysis (Iqbal, 2005).

2.3 Analysis of moisture of rice bran

The analysis of moisture was performed by weighing the sample before and after drying process by using a moisture analyser (XM 66 Precisa S/N 5600481 330XM Series). One gram of rice bran was weighed and placed on the weighing pan. The initial weight was recorded. All measurement was taken for day 0 until day 40 upon analysis.

2.4 Extraction of rice bran oil

The extraction of rice bran oil was done accordingly to the method by Amarasinghe and Gangodavilage (2001) but with modification of the equipment. The extraction was performed using the Gerhardt Soxtherm extractor (S/N: 8445-12-0027/Model.no,SE 414) at Functional Food Laboratory, Agro Biotechnology Institute, Serdang Selangor. Soxtherm is an analytical instrument to systematically extract and determine the crude oil. The principle of soxtherm is similar with soxhlets. The procedure to determine the extracted crude rice bran oil is presented in the following section.

2.5 Determination of crude rice bran oil (yields)

The yield of the crude rice bran oil is determined by the weight loss method through the loss of moisture by thermal treatment using an oven. The extraction beaker containing the extracted oil was dried in an oven for one hour at 105 °C. The dried oil was allowed to cool down in the desiccator for one hour. The weight of extraction beaker (with oil) was recorded. This step is repeated until constant weight is recorded. The recorded weight was used to calculate the crude oil yield by following Eq(1):

$$W = \frac{M_2 - M_1}{M_0} \times 100 \% \quad (1)$$

where,

W = Crude oil yields (%)

M₀ = Weight of rice bran sample (g)

M₁ = Weight of extraction beaker with boiling stone (g)

M₂ = Weight of extraction beaker with boiling stone + Oil (g)

2.6 Determination of free fatty acid

The oil that was extracted by ethanol, hexane and methanol in Soxtherm extractor for ± 4 h in total volume was recovered. The determination of free fatty acid analysis was performed according to the method described by Nasir et al. (2009), but with some modifications. The solvent from the extract was removed and the oil residue was dispersed in 75 mL of isopropyl alcohol (IPA). 25 mL of 95 % ethanol was neutralised with 0.05 N KOH which indicated by appearance of faint pink colour.

Approximately three to four drops of 0.04 % phenolphthalein were added to 95 % ethanol that had been neutralised. About 50 mL aliquots was titrated with standard 0.05 N KOH using Metrohm 848 Titrino Plus. The mixture was stirred with magnetic swing-out stirrer, autotitrator (NR. 1848001019125, Switzerland). Meanwhile a blank consisting of 50 mL with a 1:1 mixture of isopropyl alcohol and neutralised 0.04 % phenolphthalein in 95 % ethanol was titrated. The FFA content was calculated as oleic acids and expressed as THE percentage of total lipids. The calculation was carried out according to the Eq(2).

$$FFA = \frac{N \times V \times M_r \text{Oleic acid}}{W} \times 100 \% \quad (2)$$

where,

N = Normality of alkaline

V = Volume of titrant

W = Rice bran weight

M_r Oleic acid = 282 g/mol

2.7 Statistical Analysis

All the results recorded were analysed by using ANOVA and T-Test by using data analysis tool in Microsoft Excel. The value of P < 0.05 indicates significance differences between means of group studied.

3. Result and discussion

3.1 Storage stability study on unstabilised rice bran

Moisture content of unstabilised rice bran during storage is shown in Table 1. An increase of moisture content was observed during the storage because of the low temperatures (4 °C) in the chiller.

Table 1: Moisture content of unstabilised rice bran during 40 d of storage

Day	Moisture Content (%)		
	Bario	Lowland	Upland
0	0.95 ± 0.02	1.42 ± 0.12	0.95 ± 0.03
5	3.20 ± 0.01	2.26 ± 0.14	1.61 ± 0.00
10	5.97 ± 0.06	4.38 ± 0.23	4.37 ± 0.20
20	7.30 ± 0.02	6.42 ± 0.08	6.16 ± 0.07
30	7.92 ± 0.01	7.38 ± 0.00	6.87 ± 0.12
40	8.57 ± 0.10	7.46 ± 0.12	7.30 ± 0.03

Table 1 shows that the moisture content was increasing throughout the 40 d of storage even at low temperatures, indicating their high instabilities. Increase in moisture content of the rice bran is not favourable as it will hinder the efficiency of storage, application and transportation of the rice brans to its determined destination, which can lead to significant economic loss.

The milling process is highly disruptive; hence it is really challenging to avoid the rapid chemical and physical degradation of rice bran in order to preserve its quality at the highest level. The variability of rice bran is mainly due to the relatively high levels of lipase activity in raw rice bran. In intact rice kernel, rice bran lipase is substantially separated from lipid. Lipase is contained in the cross-testa layer of the kernel whereas lipid is located in the aleurone and sub aleurone layer and germ (Luh, 1991). When the bran is removed from the kernel, rice surface is disturbed and the lipid and lipase are brought together.

Rice bran is reported to contain 2 - 4 % free fatty acids (FFAs) of lipid of intact bran (Orthofer, 1996). However, after milling, free fatty acids increase rapidly. The hydrolysis of lipid to free fatty acids outcomes in high lipid loss during storage and oil refining problems when rice bran is not processed immediately at the place where the rice bran is milled. Lipoxygenase and peroxidase also affect oxidative stability of rice bran. The oxidative instability is responsible for rancid flavour and off odour of rice bran (Champagne, 2004). Stabilisation of rice bran is therefore required to inactivate these active enzymes. The change in fatty acid composition rice bran was observed within the direct solvent extraction during storage. The fatty acid compositions of the total oil yields extracts from oil recovered from rice bran stored at 4 °C are shown in Table 2 and 3.

Table 2: Oil yields percentage upon 40 d storage in 4 °C

Days	Oil yields (%)		
	Bario	Lowland	Upland
0	13.4 ± 0.03	16.2 ± 0.22	13.8 ± 0.02
5	12.3 ± 0.01	15.8 ± 0.13	12.0 ± 0.12
10	10.1 ± 0.00	14.2 ± 0.06	11.0 ± 0.09
20	9.56 ± 0.04	12.5 ± 0.04	10.0 ± 0.13
30	7.64 ± 0.00	9.55 ± 0.00	9.01 ± 0.18
40	6.56 ± 0.00	7.89 ± 0.12	8.87 ± 0.07

Table 3: Free fatty acids content percentage upon 40 d storage in 4 °C

Days	Free fatty acids content (%)		
	Bario	Lowland	Upland
0	2.50 ± 0.31	2.80 ± 0.09	2.48 ± 0.42
5	2.82 ± 0.02	3.76 ± 0.01	2.66 ± 0.13
10	4.55 ± 0.02	6.89 ± 0.05	4.87 ± 0.56
20	6.68 ± 0.04	8.89 ± 0.05	7.77 ± 0.41
30	8.89 ± 0.00	10.0 ± 0.21	9.98 ± 0.23
40	10.1 ± 0.39	12.7 ± 0.09	11.9 ± 0.22

The study is correlated with the rice bran storage under low temperature. The rice bran was extracted with a solution where the methanol was used as the solvent. Bario type shows decrease of oil yielding from 0 - 40 d with different of 48.9 %, followed by lowland types by 48.7 %. Upland rice bran oil gives the higher different percentage by 64.2 % during the storage. Free fatty acid contents show increase amount with the percentage increment for bario 7.6 %, lowland 9.9 % and Upland 9.4 %. Results from both table indicated that decrease in oil yields leads to increase in free fatty acid contents along the 40 d of storage time, indicating continuous hydrolysis and oxidation of the oil in the rice bran to free fatty acids, even at low temperature.

3.2 Effect of microwave-assisted stabilisation on moisture content of rice bran during storage

The change in fatty acid percentage in the rice bran become increasing apparent with the increase of storage time. The fatty acid increase significantly ($P < 0.05$) up to 22 % of the original value after storage for 40 d. In contrast, the oil yields significantly decreased ($P < 0.05$) by about 48 - 62 % as shown in Table 1. The result of the fatty acid composition indicated that the rice bran lipases preferentially cleaved fatty acids than other fatty acids (Takano, 1993).

Figure 1 shows the relationship of the stability of rice bran with its moisture content. The stabilised rice bran showed increment of moisture content from average 1 % - 8.57 % for bario, 1.4 % - 7.46 % for lowland and

0.95 % - 7.3 % for upland rice bran. This result agreed with reports by Jayaraman et al. (1994) where moisture content changes in rice bran during storage. It is a concern that the free fatty acid changes are deterioration by increasing the time of storage.

The increase of moisture in unstabilised rice bran with different types of rice was reported in this study. The increment of moisture was about 8 % up to 24 % in the 40 d of time of storage. The result indicates the differences in stability between the unstabilised (raw) and stabilised (treated) rice bran. The moisture content in the treated rice bran remains fairly stable across the 40 d of time of storage. The increase in moisture content in the stored rice bran is not favourable as this may lead to further degradation and spoilage, which costs significant economical lost.

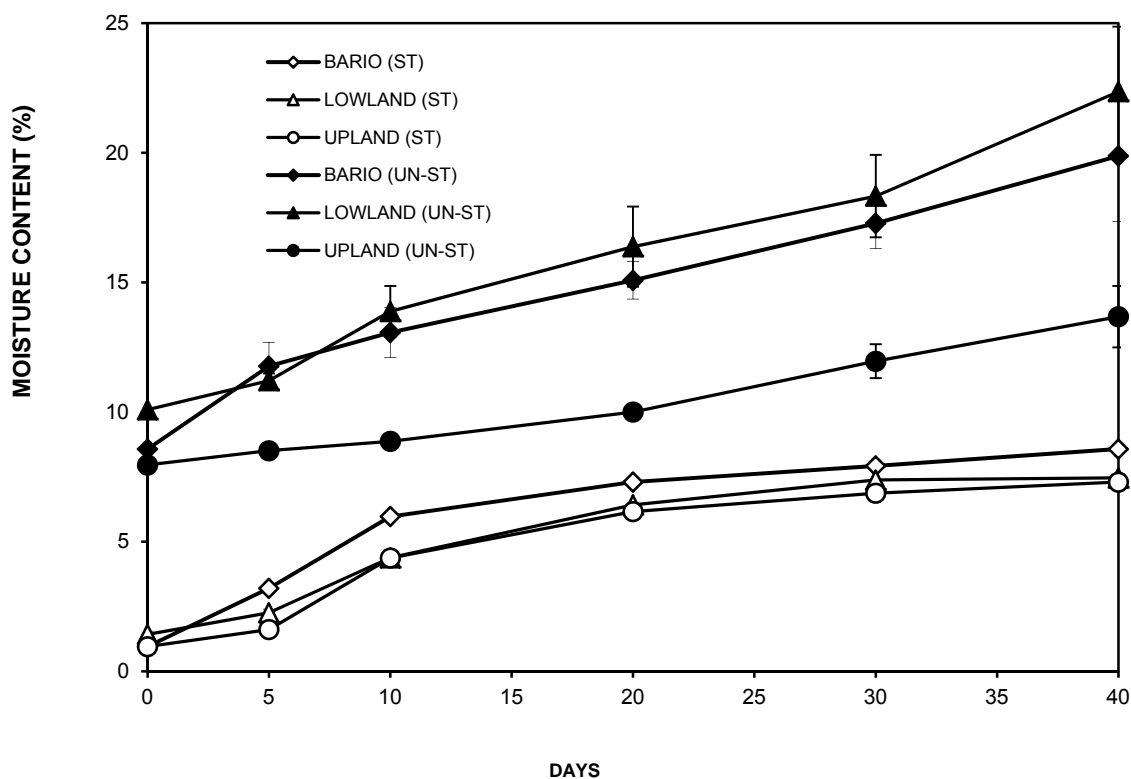


Figure 1: Moisture content of stabilised (ST) and unstabilised (UN-ST) rice bran from different varieties within 40 d storage

Treated rice bran such as parboiled rice bran was reported to be more stable and can be stored longer than the unstabilised rice bran (Jayaraman et al., 1994). Even though the parboiled and microwave technique are the two different methods of stabilising the rice bran, it can be comparable as both technique promotes the heat transmitted to the samples.

The major lipid class of oil from freshly milled rice bran is triacylglycerol. The changes of each lipid fraction of oil bodies followed a similar trend to the rice bran during the period of storage. The amount of triacylglycerol in oil decreased while that of free fatty acids increased. The triacylglycerol mobilisation was observed concurrently with the gradual decrease of yields as shown in Table 2. This indicated that the main components of the oil were damaged during the storage of rice bran.

4. Conclusion

In this study, the stability of three different rice brans, namely bario, lowland and upland rice, was compared between their stabilised and unstabilised forms on the following properties: the moisture content, the yield of extracted crude oil from the rice bran and the content of free fatty acids, along a storage period of 40 d. The storage stability study shows the production of low oil yields decreases with an increasing of FFA content along the duration of storage. As the storage time increases, more oil in the rice brain will be hydrolysed and oxidised to FFA, which is unsuitable for consumption. The stabilisation of rice bran is critical to maintain the stability of rice bran for long storage time. This can be done by reducing the moisture content in the rice bran. By decreasing the temperature and its relative humidity during the storage of rice bran, it will increase the rate

of lipid hydrolysis (hydrolysis of FFAs) and oxidative rancidity (changes of lipid hydroperoxide). Based on the characteristics of some of the rice bran studied, it can be concluded that the homogeneity of the moisture distribution determines the free fatty acids content. By controlling the moisture distribution and the related factors, it should successfully reduce the percentage of free fatty acid that can be oxidised in the rice bran oil. The oxidation of free fatty acid is unsuitable for consuming. From this study, it is concluded that the microwave stabilisation method significantly improved the properties of rice bran by reducing the moisture content during storage.

Acknowledgments

Authors acknowledge Universiti Putra Malaysia, Serdang for facilities and laboratory instrument and Faculty of Chemical Engineering, Universiti Teknologi Malaysia for the equipment and supports. The research was funded by Fundamental Research Grant R.J130000.7844.4F447.

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